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Impact of compost agronomic application on soil chemical properties and olive trees (*Olea europaea* L.) growth parameters

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Abstract

The effects of compost agronomic application were evaluated in a Tunisian olive grove. Two treatments were selected: The first plot was spread by 5 T ha^{-1} of compost and the second plot was not amended, and served as a control during the crop season 2018/2019. This study aimed to investigate soil chemical properties, plant growth parameters, photosynthetic pigments, leaf mineral nutrient and olive oil quality of adult Chemlali olive trees subjected to compost application. The findings revealed an improvement of organic matter (39.8%), total nitrogen (41.3%), available phosphorus (24.5%) and exchangeable potassium (25.1%) soil contents, in amended plot with waste date compost compared to the control in superficial horizon (0-20 cm). In fact, a vegetative activity improvement according to the compost rate spread was noted with a shoot length increment equal to 20.42% in olive trees amended by compost. Moreover, significant raise of photosynthetic pigments contents in compost amended olive trees was noticed. The compost spreading significantly increased the mineral contents of leaves (N, P, K, Ca and Mg). Finally, the oil quality parameters did not show any significant difference. With the only exception of the phenol content, this was significantly higher in the oils extracted from compost amended olive trees than the control.

Keywords: Compost, soil, chemical parameters, olive trees, photosynthetic pigments, leaves mineral contents, oil quality.

1. Introduction

Agricultural practices, based on periodical organic amendment inputs are strongly recommended for poor soils, especially those of Mediterranean agro-ecosystems. Composting was considered as an effective biological, economical and sustainable process to reuse the organic matter wastes (Muscolo et al., 2018; Doña-Grimaldi et al., 2019). Indeed, the composting process stabilizes the organic wastes by their conversion into humic substances and inactivates pathogen flora, allowing compost use for soil amendment (Zhang and Sun, 2015). Composts prepared from different agro-industrial by-products are commonly used as organic fertilizers (Ibrahimi and Gaddas, 2015). The compost application can potentially affect the soil organic matter levels and nutrient status. As a result, compost use has received great attention from agriculturists and environmentalists because of direct and indirect effects on the soil properties improvement, as well as on the plants growth and yields (Toumpeli et al., 2013). Olive grove soils are typically Mediterranean, characterized by low organic matter content, by the semiarid climate in which they develop, and degradation by human activities (Nieto et al., 2010). Olive grove soils there fore typically showless than 1% in organic carbon especially in arid regions (Chehab et al., 2019). In this context, increasing soil organic matter becomes imperative. Indeed, when applied in field trials as an organic additive, compost can improve the soil physico-chemical properties which affect positively the plant yield (Boutchich et al., 2018).

Application of compost as amendment that contribute effectively to nutrient supply for plant growth is therefore of interest, and can also improve inherent physical soil properties and preserve soil functions altered by agronomic practices (Aranda et al., 2015).

Therefore, in the present study, an industrial ecological interest was considered in the date palm waste' valorization by its co-composting with goat manure, producing soil amendment for agriculture fields. The aim of the present investigation was to study the effects of compost on soil chemical properties, olive trees growth parameters and oil quality under natural field conditions.

2. Materials and methods

2.1. Field investigation

In the present study, the experiments were carried out in a rain-feed olive grove (*Olea europaea*, L., variety Chemlali) at the Taous experimental station of the Olive Tree Institute of Sfax, in central Tunisia (34°43' N, 10°41' E). This area of the country has a typical Mediterranean climate, with an average annual rainfall of about 200 mm.

The field was divided into two plots. The first plot was spread by 5 T ha^{-1} of compost and the second plot was not amended, and served as a control during the crop season 2018/2019. Each of the two plots covered an area of 1 hectare and contained 16 eighty-year old trees, with an inter-tree spacing of 24 m × 24 m. The experimental orchard was characterized by a sandy soil (86.63% sand, 13.26% silt and 0.20% clay) (Magdich et al., 2020).

2.2. Compost used for experimentation

The compost used in this investigation was prepared by mixing goat manure (1/3) with crushed date palm waste fibers (2/3) and applied on the soil surface by mechanical spread. The crushed date palm waste (*Phoenix dactylifera* L.) composed essentially of palm leaflets and the goat manure, both collected from the Oasis of Cheneni-Gabes (South of Tunisia) were used for the composting procedure carried out at the Chenini Oasis Protection Association composting site (E: 10°04'40''; N: 33°53'01''). Date palm residues were mechanically crushed into peace of 5 mm in diameter. Then, they were hydrated in basin to improve their biodegradability during the composting process. The raw materials were mixed at a proportion of 2/3 crushed-hydrated palm waste (v:v) and 1/3 manure. These wastes were co-composted in an open area, using a windrow of 10 m length, 1.5 m wide base and 1.3 m height. These materials were mixed by mechanical rotation allowing aeration (Abid et al., 2020). The compost physico-chemical properties are mentioned in Table 1.

2.3. Soil chemical analysis

The pH and electrical conductivity (EC) were determined on a mixture of soil/water (1:2.5 and 1:5, respectively). The soil organic matter (OM) was analysed according to the Walkley-Black method (Nelson and Sommers, 1996). Total nitrogen content was determined by the Kjeldahl method. Available phosphorus was analysed following the method of Olsen and Sommers (1982). Available K^+ and Na^+ were extracted with ammonium acetate at pH 7. Then, they were determined by atomic absorption spectrophotometry (Hitachi U-2000).

Table 1. Compost physico-chemical composition
Values represent means of three replications ± SE

Parameters	Values
pH	7.50 ± 0.14
Electric conductivity (mS cm^{-1})	7.74 ± 0.06
Moisture (%)	17.52 ± 0.45
Organic matter (%)	54.84 ± 0.62
Total organic carbon (%)	29.26 ± 0.60
Total Kjeldahl nitrogen (%)	1.86 ± 0.22
C/N	15.73 ± 0.60
Mineral matter (%)	45.16 ± 0.42
P (g kg^{-1})	10.24 ± 0.54
K (g kg^{-1})	87.60 ± 0.42
Ca (g kg^{-1})	49.60 ± 0.62
Mg (mg kg^{-1})	230.23 ± 0.89
Na (mg kg^{-1})	136 ± 0.26
Germination index (%)	88 ± 0.40

2.4. Growth parameters

At the beginning of the growing season (March 2019), four shoots per plants × 4 plants per treatment for every treatment were selected and tagged. Ten days before the compost application, the length of the main-shoot (in cm) and the number of the leaves were measured. These measurements were made periodically every fifteen days.

2.5. Total chlorophyll and carotenoid concentrations

For the photosynthetic pigments content determination, leaf discs for each treatment were taken from five fully expanded leaves, collected and transferred immediately to the laboratory for analysis. Leaf sections were ground in 80% acetone. The total chlorophyll and the carotenoid concentrations were determined spectrophotometrically according to the method of Lichtenthaler (1987).

2.6. Leaf nutrient concentrations

In order to determine the leaf mineral contents, leaves samples were over-dried at 70 °C for 48 h and then ground to a fine powder. A 1 g representative mass of the fine powdered sample was dry-ashed in a muffle furnace at 450 °C for 6 h. Then, the ash was dissolved in HNO_3. The total N was analysed using the Kjeldahl method. The concentrations of the mineral elements K, Ca and Mg were determined by atomic emission spectrophotometry (Walinga et al., 1989). The P was determined by a vanado-molybdate colorimetric procedure (Magdich et al., 2016).

2.7. Olive oil analysis

2.7.1. Oil extraction

The harvested olives were immediately transferred to the laboratory where the randomly collected olive samples from the same plot were homogenised and a representative sample (in total 6 kg of olive fruits per treatment) was used for oil extraction according to the International Olive Council legislation method specific for the extra virgin olive oil (IOC, 2015). Olive oil used for analysis was extracted using a laboratory olive Bench Hammer Mill (Abencor Analyzer, MC2 Ingenierias y Sistemas, Sevilla, Spain). After fruit crushing and paste mixing for 30 min at 25 °C, the centrifugation allowed the olive oil separation. The oil extraction yield was determined. Oil samples were filtered, transferred into amber glass bottles, and stored at 4 °C in darkness until analysis.

2.7.2. Oil concentration

Fruits samples were dried in an oven at 80 °C to a constant weight. Oil concentration, expressed as a percentage on dry olive paste weight basis, was determined by Soxhlet extraction with hexane at boiling point of the solvent as previously described by Allalout et al.
(2009).

2.7.3. Oil quality parameters

a. Chemical oil quality indices

Extinction coefficients K_{232} and K_{270} were measured at 232 and 270 nm, respectively. Free acidity and peroxide value, expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2 kg^{-1}), were measured according to the analytical method described in the European Regulation EEC 2568/91.

b. Chlorophyll and carotenoid concentrations

The chlorophyll and carotenoid concentrations were evaluated from the absorption spectrum at respectively, 670 nm and at 470 nm of each olive oil sample (7.5 g) dissolved in cyclohexane, as described by Mínguez-Mosquera et al. (1990).

c. Total phenols content

Total phenol content was determined using Folin-Ciocalteu's colorimetric analysis. Phenolic compounds were extracted from 2.5 g of olive oil dissolved in 5 ml hexane using a solution of methanol and water (5 ml; 60/40, v/v). The mixture was then vigorously shaken for 2 min. Folin-Ciocalteu reagent (0.5 ml) and bi-distilled water (4.8 ml) were added to the phenolic fraction. In order to have a final volume of 10 ml, a solution of sodium bicarbonate (1 ml; 35%, w/v) and an amount of water were added. The mixture was incubated for 2 h in the dark at room temperature. The absorbance was measured at 725 nm and expressed as parts per million (ppm) of gallic acid (Montedoro et al., 1992).

2.8. Statistical analysis

The cumulative data recorded were subjected to variance analysis using SPSS Statistics 20.0 for Windows. The treatments mean values were compared using Duncan's multiple range test at the 5% ($P = 0.05$) significance level.

3. Results and discussion

3.1. Compost characteristics

The compost derived from date palm waste and goat manure co-composting had a neutral pH, a relatively high electrical conductivity (EC) (Table 1). Its relatively low C/N ratio and high germination index value reflected its stability. Considering its total nitrogen content and organic matter (OM) rate inferior to 3% and 55% respectively, the used compost could be further classified as green waste compost according to French standards (NF U44-051, 1980). Its high macro and micronutrients concentrations could be useful to improve soils and are comparable than those reported by other previous studies (Muscolo et al., 2018).

3.2. Compost impact on the soil chemical characteristics

According to table 2, the soil pH at surface horizons (0-20 and 20-40 cm), noted to significantly decrease in comparison to the control ($P < 0.05$), following the amendment of compost. This could was be attributed to NH_4^+,

CO_2 and organic acids production during the compost organic matter mineralization by the microbial communities (Garcia-Ruiz et al., 2012). Similarly, the compost induced an increase in the EC of the soil-layers compared to the control soil. This result was attributed to the compost EC which was 7.74 mS cm^{-1}. This result is in agreement with several investigations which have mentioned soil EC raises following the application of organic amendments especially compost (Franco-Otero et al., 2011; Aranda et al., 2015). OM contents increases were noted in all the soil horizons after the compost spreading in the olive field. This OM enhancement was more accentuated at the surface layers (0-20 and 20-40 cm) and the content decrease was observed with the depth. These results are in accordance with several reports in the literature (Chaker et al., 2019, Masmoudi et al., 2020). In this perspective, Ouni et al. (2013) showed that palm waste compost leads to soil OM increases, attributed especially to its high OM content. In addition, Mekki et al. (2014) found that agro industrial compost application in an arid climate improves significantly the soil OM content. Moreover, the different soil layers analysed were noted to undergo significant improvements of soil K, N and P contents with compost application (Table 2).

Table 2. Soil chemical parameters at the different layers in the experimented field after compost application Values represent the means of three samples (± SE).

Treatments	Layer (cm)	pH	Electrical conductivity (mS cm^{-1})	Organic matter (%)	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Na (mg kg^{-1})
Control	0-20	7.87 ± 0.02	0.34 ± 0.05	0.68 ± 0.02	211.50 ± 1.56	42.45 ± 2.23	128.00 ± 2.30	95.00 ± 1.56
	20-40	7.96 ± 0.06	0.32 ± 0.03	0.48± 0.03	161.00 ± 0.23	36.65 ± 2.78	121.00 ± 3.24	80.00 ± 2.84
	40-60	7.03 ± 0.04	0.27 ± 0.04	0.36 ± 0.02	67.00 ± 3.45	31.22 ± 2.65	78.00 ± 3.23	78.00 ± 2.37
	60-80	7.97 ± 0.03	0.29 ± 0.02	0.31 ± 0.01	47.00 ± 1.47	29.62 ± 3.42	59.00 ± 2.78	60.00 ± 1.24
Compost: 5 T ha^{-1}	0-20	6.91± 0.02	0.85 ± 0.03	1.13 ± 0.04	360.50 ± 1.42	56.25 ± 2.98	171.00 ± 2.75	116.00 ± 1.72
	20-40	6.96 ± 0.03	0.75 ± 0.02	1.04 ± 0.02	241.50 ± 0.78	51.38 ± 3.52	131.00 ± 1.87	100.00 ± 1.53
	40-60	7.41 ± 0.05	0.67 ± 0.03	0.55± 0.01	111.50 ± 2.45	28.27 ± 3.42	91.00 ± 0.23	94.00 ± 1.46
	60-80	7.26 ± 0.02	0.55 ± 0.02	0.42 ± 0.03	78.00 ± 1.36	23.12 ± 1.94	87.00 ± 2.24	75.00 ± 0.45

The highest increase of these contents was recorded particularly at the soil upper layers (0-20 and 20-40 cm). In this context, Masmoudi et al. (2020) showed a total nitrogen improvement of the soil contents when using heterogeneous composition compost. Also, Aranda et al. (2015) mentioned the potassium content increase in soil layer (0-10 cm) amended with olive-mill pomace co-compost after 17 years of amendments. Concerning, the soil sodium content, an increase was noticed with the compost treatment without a significant difference compared to the control ($p > 0.05$).

3.3. Plant Growth parameters

After one year of compost spreading, vegetative activity (evaluated as shoot length and leaves number) was higher in the olives trees subjected to organic fertilisation compared to the non-treated olive trees (Figure 1).

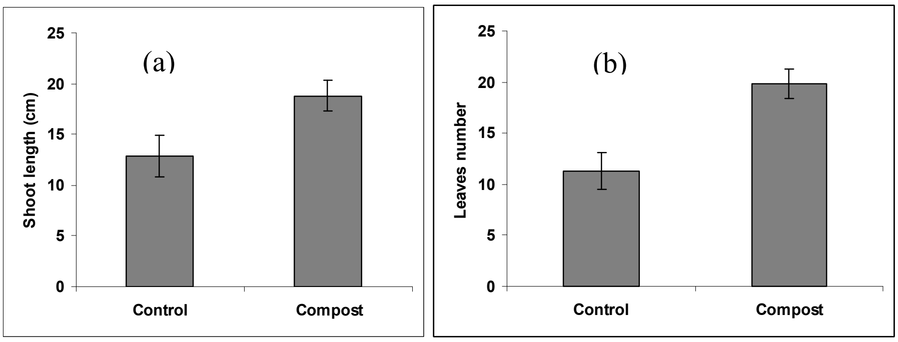

Figure 1. Shoot length (a) and leaf number per shoot (b) of olive trees subjected to compost application at different rates (control: 0 T ha^{-1} and 5 T ha^{-1}). Vertical bars represent means of 3 replications ± S.E. and bars with different letters are significantly different ($P < 0.05$).

The shoot length increment was equal to 20.42% in olive trees amended by compost. The vegetative activity improvements could be related to the compost richness in easily assimilated available substances, providing carbon, nitrogen, phosphorus, and potassium to soil (Głąba et al., 2020). The positive effect of compost amendment on olive growth has been also demonstrated by Fernández-Hernández et al. (2014) in olive orchard amended over 6 years. Similar results have been also recorded in the case of *Tagetes erecta* plants amended with compost by Shafique et al. (2021). Thus, Abid et al. (2018) exhibited that date palm compost improved the plant root elongation rate when used as an additive to the different substrate compositions.

3.4. Photosynthetic pigments

The Chemlali olive plants which received 5 T ha^{-1} of compost, showed higher photosynthetic pigment concentrations when compared to the non-amended olive trees (Table 3).

Table 3. Total chlorophyll (a + b) and carotenoid contents of Chemlali olive leaves

Treatments	Chlorophyll (a + b) (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
Control	11.66 ± 0.46 ^{a}	2.46 ± 0.12 ^{a}
5 T ha^{-1} year^{-1} of compost	14.21 ± 0.75 ^{b}	2.78 ± 0.16 ^{b}

Values represent the means of three samples (± SE). Different letters (a-b) indicate significant differences ($P < 0.05$) between treatments treated separately.

The chlorophyll (a + b) and carotenoid increase were 17.94% and 11.51% respectively by comparison to the control. Indeed, the photosynthetic pigments increment registered in olive trees subjected to compost exhibited photosynthetic performances improvement following this organic application. In this perspective, Chehab et al., (2019) mentioned a photosynthetic pigment contents raise of olive tress cv. Chemlali following a compost spreading constituted by: sheep manure (40%), olive pomace (40%) and olive tree pruning (20%). In this perspective, Bashir et al. (2020) noticed an improvement in chlorophyll contents in wheat leaves after compost application.

3.5. Leaf nutrient content

The olive-tree leaves mineral contents (N, P, K, Ca and Mg) were affected by the compost agronomic application (Table 4). Indeed, leaf nutrient analysis of the olive trees amended with 5 T ha^{-1} of compost, showed significantly higher concentrations than that of the control ($P < 0.05$). This mineral rise was attributed to the soil chemical properties improvement as which organic matter, total nitrogen, phosphorus and potassium contents. In this context, Fernández-Hernández et al. (2014) showed an olive leaf nitrogen content raise following the various mature composts addition to a typical ‘‘Picual’’ olive tree grove in the Jaén province (Spain) during six consecutive years. Similarly, Chehab et al., (2020) noticed a significant potassium concentration increase of olive trees amended by compost applied at the experimental farm of the Professional Agricultural School in Jemmel-Provence of Monastir-Tunisia.

Table 4. Leaf mineral nutrient content (% DM) after compost agronomic application Means followed by a different letter within a row are significantly different at $P < 0.05$ according to Duncan’s multiple comparison test

Treatments	N	P	K	Ca	Mg
Control	1.812 ± 0.004 ^{a}	0.080 ± 0.002 ^{a}	0.872 ± 0.004 ^{a}	1.722 ± 0.002 ^{a}	0.102 ± 0.002 ^{a}
5 T ha^{-1} year^{-1}	1.887 ± 0.003 ^{b}	0.089 ± 0.005^{b}	1.116 ± 0.005 ^{b}	1.799 ± 0.005 ^{ab}	0.160 ± 0.004 ^{ab}

3.6. Fruit oil concentration

The compost amendment has led to a significant increase of oil accumulation in the Chemlali olive tree variety in comparison to the control (Table 5). As a result, the date palm waste' compost seems to affect positively the growth and rather than the oil biosynthesis mechanism. Interestingly, the significant differences in oil content between the control and the compost treatment ($P <$ 0.05) would present an advantage of this organic amendment for the studied cultivar, and its potential role as a fertilizer. In this perspective, Fernández-Hernández et al. (2014) noted the significant increase in olive oil content after compost application in olive trees orchard.

Table 5**.** Total oil content, free acidity, peroxide value, extinction coefficients, total chlorophylls and carotenoids in the olive oil samples from Chemlali plants subjected to compost application during the crop season (2018/2019)

	Control	5 T ha^{-1} year^{-1}
Total oil content (% FW)	24,25 ± 2.66 ^{a}	28,56 ± 1.46 ^{b}
Free acidity (%)	0,26 ± 0.01 ^{a}	0,28 ± 0.01 ^{a}
Peroxyde value (meq O_2 kg^{-1})	2,45 ± 0.03 ^{a}	2,60 ± 0.03 ^{b}
K 232	1,98 ± 0.03 ^{a}	2,06 ± 0.04 ^{a}
K 270	0,17 ± 0.02 ^{a}	0,18 ± 0.02 ^{b}
Total chlorophyll (mg kg^{-1})	0,34 ± 0.05 ^{a}	0,32 ± 0.04 ^{a}
Carotenoids (mg kg^{-1})	1,86 ± 0.04 ^{a}	1,72 ± 0.02 ^{a}
Total phenols (mg kg^{-1})	108,54 ± 2.24 ^{a}	146,14 ± 3.35 ^{b}

Values represent the means of three samples (± SE). Different letters (a-b) indicate significant differences ($P <$ 0.05) between regions treated separately.

3.7. Olive oil quality parameters

The oil analyzed samples collected from the two different treatments were classified as “extra virgin olive oil”, according to the International Olive Council (IOC, 2015) norms for quality indices (oleic acidity ≤ 0.8%, peroxide value ≤ 20 meq O_2 kg oil^{-1}, K270 ≤ 0.22 and K232 ≤ 2.5) (Table 5). Therefore, the compost fertilisation did not affect the free acidity, the peroxide value and the extinction coefficients of the produced oils since no statistically significant difference ($P >$ 0.05) was recorded between the control and the compost used for soil amendment. Furthermore, the total phenols contents showed an increase in oil samples issued from compost amended plants. This increment was equal to 25.7% compared to the control and is of great interest, since The phenol content increases in the olive oil from the compost amended plot is an interesting result because polyphenols are not only associated to the nutritional and sensory olive oil qualities, but also play a beneficial role in human health by their anticarcinogenic, antiatherogenic, antimicrobial and antioxidant activities (Ben Youssef et al. 2012; Galanakis and Kotsiou 2017).

These findings confirm Proietti et al. (2015) conclusions, who claimed solid oil mill waste and its derived-compost application to the soil for three consecutive years in an olive grove did not lead to a negative impact on oil quality with a phenol content increase. In addition, Chehab et al. (2019) reported that compost application in olive orchard had no negative effect on oil quality parameters with a phenol content increase.

4. Conclusion

The organic fertilisation of date palm waste' compost influenced the soil parameters and the adult Chemlali olive trees. The compost application was accompanied by an increases in organic matter, total nitrogen, phosphorus and potassium contents in all soil layers investigated (0-80 cm), which have positive effects on soil fertility and stability. Thus, an improvement in chlorophyll (a + b) and carotenoid contents and leaf mineral nutrient were evidenced for olive plants amended by 5 T ha^{-1}. Compost spreading improved the vegetative olive tree activity and the oils issued from the olive trees subjected to compost application were classified in extra virgin olive oil category. Further investigations should be conducted to study the long-term effects of this sustainable soil management strategy on soil microbial activity and on olive tree physiology and productivity.

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Fatty acid and Antioxidant content of Chemlali Extra Virgin Olive Oil and its Hydrophilic and Lipophilic Fractions

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Abstract

Olive Oil is a fundamental ingredient of the Mediterranean diet for its healthy properties.

The aim of this study was to determine i) the most relevant quality parameters (Free acidity, K232, K270 and PV) of *chemlali* olive oil samples ii) to evaluate fatty acids and antioxidant content of *chemlali* olive oil and its hydrophilic (OOHF) and lipophilic (OOLF) fractions iii) to compare the chemical characterization between olive oil and its fractions. OOHF was extracted from olive oil using water by centrifugation process. OOLF was obtained by filtration process through a hydrophobic composite ceramic membrane. According to quality parameters, all our oil samples were classified in the category "extra virgin olive oil". Our results showed that EVOO and OOLF fatty acid analyses revealed the same amount of MUFA. EVOO and OOHF contained a higher content of unsaponifiable components like polyphenols, which might contribute to olive oil's beneficial effect.

Keywords: Extra Virgin olive oil, hydrophilic fraction, lipophilic fraction, antioxidant content, Fatty acid composition.

1. Introduction

The olive oil sector plays an important role in the Tunisian economy, providing both employment and export revenue. The olive tree (*Olea europaea* L.) is present practically in every region of the country, up to the border of the southern desert. The Tunisian olive grove is dominated by the two major varieties Chetoui in the North and Chemlali in the Centre and the South. Olive oil is composed of two major fractions, the lipophilic one, accounting for 98-99% of the total triglycerides, and hydrophilic fraction, containing several liposoluble molecules, including tocopherols, phytosterols, coloring pigments and squalene (Bulotta *et al*., 2014; Wu et al., 2016).

The main antioxidants of extra virgin olive oil (EVOO) are carotenoids and phenolic compounds, which are both lipophilic and hydrophilic. Among the glyceride fraction, olive oil shows a high content of fatty acids and particularly, an elevated proportion of monounsaturated fatty acids (MUFA). Unsaturated acids are up to 85% of its composition, due to its high content in oleic acid (C18:1), which might range between 70–85% and other fatty acids as linoleic or palmitoleic acid. Olive oil is considered as a superfood due to its health properties derived from its unique composition, its lipid profile and its bioactive compounds content (Secneler and Galanakis, 2019).

MUFA can modulate the immune response and can be useful in treating certain autoimmune diseases and in general regulation of immunity (Miles and Calder, 2015). Moreover, chronic diseases such as coronary arterial disease, hypertension, diabetes, inflammatory and auto immune disorders can be prevented via olive oil intake due to in part to the high MUFA content (Bermudez *et al*., 2011). The important element that contributes to the accumulation of the intracellular reactive oxygen species (ROS) is the endoplasmic reticulum (ER) stress (Malhi *et al*., 2011). Hydroxytyrosol (3,4-dihydroxyphenylethanol; HT) is mainly responsible for the antioxidant properties of this food, due to an efficient scavenger activity (Del Monaco *et al*., 2015). It has been reported that HT is able both to modulate an adaptive signaling pathway activated after ER stress and to ameliorate ER homeostasis (Giordano *et al*., 2014). In recent years, the interest of scientists has been focused on the preventive effects of phenols against the degenerative diseases mediated by ROS.

Several studies have emphasized the importance of a regular use of olive oil in the benefits of traditional Mediterranean diet on cardiovascular diseases (Sofi *et al*., 2013; Ghorbel *et al*., 2015). The aim of this study was to determine the fatty acid and antioxidant content of chemlali virgin olive oil and its hydrophilic and lipophilic fractions and to evaluate their protective effects on rats intoxicated with dietary contaminants in the second step.

2. Materials and methods

2.1. Oil samples

Biologic Extra virgin olive oil (EVOO) samples were obtained from a Chemlali variety cultivar grown in Sfax of Tunisia. The hydrophilic fraction (OOHF) was extracted from EVOO by the method of Montedoro *et al*. (1992) using water instead of methanol to avoid its toxic effect in rats. Briefly, 10 g of EVOO was homogenized with 10 mL of water by a mixer (Ultra-Turrax T25 [IKA Labortechnik, Janke & Kunkel, Staufen, Germany]; 15 000×g/min) and centrifuged at 5000 × g for 10 min. The extraction was performed two times.

2.2. Quality indices

Free acidity (g oleic acid/100 g olive oil), peroxide value (PV) (meq O_2/kg of olive oil) and the UV absorption for the determination of the extinction coefficients of K232 and K270 were measured following the analytical methods described in regulation EEC/2568/91. All parameters were determined in triplicate for each sample.

2.3. Filtration process and membrane analysis

The lipophilic fraction (OOLF) was obtained from EVOO as follows. EVOO was homogenized for 1 min with water (1:1, v/v), and the oil was separated by centrifugation; this procedure was repeated six times. Then, the OOLF was filtered through a hydrophobic composite ceramic membrane prepared totally from the phosphate industry sub product material. The Cross flow experiments were conducted using a pilot plant made in our laboratory using single channel tubular membrane at a temperature of 25◦C (Figure 1).

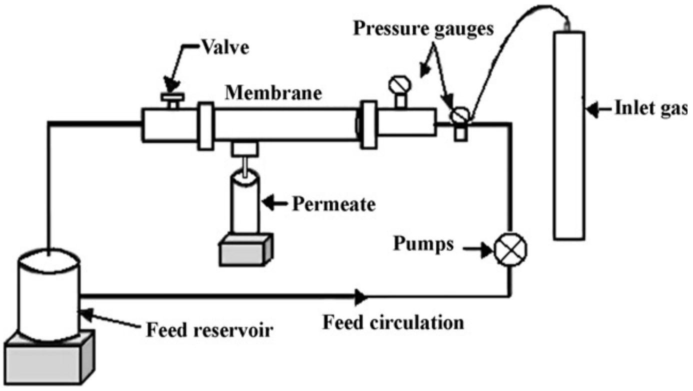

Figure 1. Scheme of the pilot plant

The operating pressure is applied using a nitrogen gas source. The total active area of the membrane is 19.6 cm^2. Before experiments, the elaborated membrane is conditioned by immersion in pure deionized water for at least 24 h, then, the membrane permeability was determined (Table 1). This material was previously used as membrane support (Khemakhem et al., 2011).

Table1. The principle characteristics of the ceramic membrane

Characteristics	Contact angle (°)	Configuration	Surface area (cm^2)	Permeability (L/hm^2bar)
Values	160	Tubular	19.6	24

2.4. Fatty acid Determination

Different constituents of Extra Virgin olive oil and its fractions were analyzed. The fatty acids were converted into fatty acid methyl esters (FAMEs) prepared by dissolving 0.1g of EVOO in methanol and incubated for 1 hour. Individual FAMES were separated and quantified by gas chromatography using model 5890 series II instrument (Hewlett-Packard Ca Palo Alto, Calif. USA) equipped with a flame ionization detector and a fused silica capillary column HP – INNOWAX (30 m length × 0.25 mm i.d. and 0.25 µm of film thickness). The temperature was programmed to increase from 170 to 270°C at a rate of 5°C/min. Nitrogen ultra was used as carrier gas. The results were expressed as relative area percent of the total FAMEs (Dabbou *et al.*, 2009).

2.5. Pigment content

The carotenoids and chlorophylls (mg/kg oil) were determined at 470 and 670 nm, respectively, in cyclohexane using the specific extinction values according to the method of (Minguez Mosquera *et al.*, 1992)

2.6. Total Polyphenol and Tocopherol content

The phenolic compounds were extracted, estimated colorimetrically at 765 nm using the Folin-Ciocalteau reagent, and expressed as hydroxytyrosol equivalents as reported by Montedoro *et al.* (1992).

α-Tocopherol was evaluated according to the method of Gimeno *et al.* (2000). Each oil sample was diluted with n-hexane (1:10), the mixture was vortexed and 200 µl were transferred to a test tube containing 600 µl of methanol and 200 µl of internal standard (300 µg/ml). HPLC separation was carried out on a Hewlett-Packard system (Waldbronn, Germany) equipped with a HP-1100 pump, a Rheodyne model 7725 injector (Cotati, CA, USA, loop volume 20 µl), a HP-1200 M multi-array detector and a Supelcosil ODS- 2 column (150 × 4.5 mm id., film thickness 5 µm).

3. Results

3.1. Quality parameters

The quality criteria, determined at the beginning of this study in order to identify the oil category, are presented in Table 2. According to the measured parameters, all oil samples were classified in the category "extra virgin olive oil" (IOC, 2015).

Table 2. Quality parameters of *Chemlali* olive oil

Free acidity (g oleic acid /100g)	K232	K270	PV (meq O_2/kg of olive oil)
0.5 ± 0.02	2.32 ± 0.02	0.21 ± 0.01	6.0 ± 1.02

3.2. Fatty acid composition of EVOO and its OOLF fraction

Fatty acid composition of EVOO and OOHF are presented in Table 3. Extra virgin olive oil and lipohilic fraction contained respectively 20.27 and 19.81% of saturate (palmitic and stearic acids), 55.09 and 53.50% of monounsaturate (mainly oleic acid), 16.12 and 15.27% of polyunsaturate fatty acids.

Table 3. Fatty acid composition of EVOO and OOLF fractions
PUFA: polyunsaturated fatty acid; MUFA: monounsaturated fatty acid; SFA: saturated fatty acids

Fatty acid (%)	EVOO	OOLF
Palmitic acid (C16:0)	17.60 ± 0.15	17.22 ± 0.12
Palmitoleic acid (C16:1w7)	2.12 ± 0.05	2.03 ± 0.03
Stearic acid (C18:0)	2.23 ± 0.04	2.15 ± 0.01
Oleic acid (C18:1w9)	52.78 ± 0.51	51.69 ± 0.12
Linoleic acid (C18:2w6)	15.44 ± 0.60	14.62 ± 0.08
Linolenic acid (C18:3w3)	0.68 ± 0.02	0.65 ± 0.05
Arachidonic acid (C20:0)	0.44 ± 0.01	0.44 ±0.02
Gadoleic acid (C20:1w-9)	0.19 ± 0.01	0.18 ± 0.02
SFA	20.27 ± 0.11	19.81 ± 0.14
MUFA	55.09 ± 0.59	53.90 ± 0.74
PUFA	16.12 ± 0.63	15.27 ± 0.22
MUFA/PUFA	3.41 ± 0.11	3.53 ± 0.04

3.3. Chlorophyll, carotenoid and antioxidant content of EVOO, OOHF and OOLF

Antioxidant content of EVOO and its fractions are presented in Table 4.

Table 4. Antioxidant content of EVOO and its fractions.
ND: non determined - : absent

Antioxidant content (mg/kg)	EVOO	OOHF	OOLF
Chlorophylls	3.28 ± 0.30	-	3.45 ± 0.12
β-Carotene	5.56 ± 0.48	0.52 ± 0.11	4.66 ± 0.79
Total polyphenols	225.12 ± 5.42	168.59 ± 2.92	55.19 ± 2.51
α-tocopherol	244.66 ± 5.11	ND	235.96 ± 11.52
β-tocopherol	0.05 ± 0.01	ND	_

Chlorophylls are present in olive oils and are the responsible for its greenish coloration. Carotenes are present too in olive oil and are responsible for its yellow coloration. The values of the chlorophylls and carotenes concentrations are 3.28 and 3.45 mg/kg mg/kg for chlorophylls and 5.56 and 4.66 mg/kg for carotenes in EVOO and OOLF respectively.

In fact, EVOO and OOHF contained high amounts of phenols (225.12 and 168. 59 mg/ kg, respectively) while OOLF contain less quantity (55.19 mg/

Kg). EVOO and OOLF presented the same amount of α-tocopherol while OOHF was deprived from this component.

4. Discussion

The virgin olive oils are classified into EVOO, virgin, and lampante, according to the degree of acidity (ratio of free fatty acids to total oleic acid): ≤0.8%, ≤2%, and >2%, respectively (Mezghani Larousi *et al.*, 2016). Lower acidity values guarantee high quality oil obtained from healthy olives and under ideal conditions. Regarding oxygen, EVOO must have a peroxide index (meq O2/kg of olive oil) ≤20. Thus, a rapid hydroperoxide formation demonstrates the initiation of the oxidative reactions that precede rancidity (Elez- Martinez *et al.*, 2007). According to quality parameters, all our oil samples were classified in the category "extra virgin olive oil". All these characteristics are key factors to EVOO quality, thus the chemical composition of many health-promoting compounds, such as unsaturated fatty acids (especially oleic acid), as well as minor components such as tocopherols or phenolic compounds must be preserved. Fatty acids can be classified in saturated or unsaturated relying on the absence or presence of double bonds in their hydrocarbon chain. Dietary fatty acids upon ingestion are incorporated into biological membranes, including the mitochondrial membrane, contributing to cell structure and at the same time modulating many of its properties (Desnoyers *et al.*, 2018). Our results showed that EVOO and OOLF fatty acid analyses revealed the same amount of MUFA. Triacylglycerols constitute a big part of olive oil and a high percentage of the saponifiable fraction is constituted by MUFA (Cavahim *et al.*, 2019). The principal triacylglycerol detected in olive oil is oleic acid (52.78%, 51.69%) respectively in EVOO and OOLF, representing about half of the total triacylglycerol portion found in EVOO. Other triacyclglycerols also present are palmitic acid, linoleic acid and stearic acid (Boskou *et al.*, 2006; Arnada *et al.*, 2004).

The unsaponifiable fraction contains more than 200 compounds; among them, phenolic compounds account for 3% of the total oil composition (Lastra Romero, 2011). This fraction contributes to the specific characteristics of olive oil, such as aroma, taste, color and oxidative stability (Frankel *et al.*, 2003). EVOO is known for having a high content of antioxidant compounds with protective properties against free radicals. In our previous study, we have demonstrated that *chetoui* olive oil and its fractions reduced biomarkers of oxidative stress in the heart of rats treated with acrylamide and aluminium via their strong antioxidant activity and thereby restored the DNA integrity of myocardial cells (Ghorbel *et al.*, 2015). The main antioxidants of EVOO are carotenoids and phenolic compounds, which are both lipophilic and hydrophilic. The lipophilics include tocopherols, while the hydrophilics include flavonoids, phenolic alcohols and acids, secoiridoids and their metabolites. Various studies indicate that EVOO phenolic compounds have antioxidant, anti-inflammatory, antimicrobial activity, by modulating gene expression of proteins involved in the inflammation process, the oxidative stress resistance and in lipid metabolism (Covas *et al*., 2006; Konstantinidou *et al*., 2010). Our results showed a higher content of unsaponifiable components like polyphenols in EVOO and OOHF which might contribute to olive oil's beneficial effect.

This approach leads to the next step, testing the beneficial effects of EVOO, OOHF and OOLF against toxicity induced by dietary contaminants in rat models.

5. Conclusion

Quality parameters revealed that our oil samples were classified in the category "extra virgin olive oil". Our results showed that EVOO and OOLF fatty acid analyses revealed the same amount of MUFA. However, a higher content of unsaponifiable components like polyphenols was found in EVOO and OOHF which might contribute to olive oil's beneficial effect. Olive oil is considered as a superfood due to its health properties derived from its unique composition, its lipid profile and its bioactive compounds content.

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Grati-Kamoun, N., Lamy Mahmoud, F., Rebai, A., Gargouri, A., Panaud, O., Saar, A. 2006. Genetic diversity of Tunisian olive tree (*Olea europaea* L.) cultivars assessed by AFLP markers. *Genetic Resources and Crop Evolution* 53:265–275.

- Book

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